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A.M. Kyrychenko, G.V. Kraeva, O.G. Kovalenko

Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Acad. Zabolotny St., Kyiv, MSP, D03680, Ukraine

BIOLOGICAL CHARACTERISTIC AND IDENTIFICATION OF SOYBEAN VIRUS ISOLATED FROM DIFFERENT UKRAINE REGIONS

To examine the presence and level of viral infection, field observations of the soybean crops in the Cherkassy, Vinnitsa and Kyiv regions have been performed. It was established that the diseases in the soybean plants growing in the examined areas have been caused by two major viruses – SMV (Soybean mosaic virus) and BYMV (Bean yellow mosaic virus). The results of field observations have been confirmed using light and electron microscopy and ELISA.

K e y w o r d s: Glycine max (L), Potyviridae, soybean mosaic virus (SMV), bean yellow mosaic virus (BYMV), spread, identification.

Soybeans are grown in many parts of the world and are a primary source of vegetable oil and protein for use in food, feed, and industrial applications due to the favorable mix of organic and mineral substances in the seeds [4]. Severe damage of soybean plants and yields losses can be caused by a number of viruses that may be regionally localized.

Soybeans are affected by a large number of diseases and pests. Under natural conditions, soybeans are infected by approximately 46 viruses. Additionally, under experimental conditions, soybeans are susceptible to more than 100 viruses from different families.

Members of the family *Potyviridae* account for almost a third of the total known plant virus species [5] infecting the most economically important crops [8] and are responsible for more than half the viral crop damage in the world [9]. Soybean mosaic virus (SMV) is a potyvirus, that is, a member of the genus *Potyvirus*, the largest genus in the family *Potyviridae* (3). SMV is a major pathogen of soybeans transmitted efficiently through seed and by aphids in a non-persistent manner; yield losses due to SMV generally range from 8 to 35 %, however losses as high as 94 % have been reported [7].

The situation with viral diseases of soybeans is exacerbated by the lack of immune and resistant varieties to viruses [1].

There is a necessity for a detailed study of the relationship of viruses and cultivated soybean plants to increase their productivity and to understand such important properties of the host as immunity, resistance and ability to localize the infection. In this paper we will provide data about occurrence of virus infection of soybean varieties in Ukraine and virus biological properties.

Materials and Methods. The different ecological regions of Ukraine – Kyiv region (experimental fields of the Institute of Microbiology and Virology), Vinnitsa region (stationary fields of the Institute of Feed, Vinnitsa) and Cherkassy region (Drabiv Experimental Field of Cherkassy Institute of Agricultural and Industrial Production) have been investigated to explore the distribution and properties of virus diseases of soybean in these areas. The degree of injury was determined by taking into account the disease symptoms in accordance with [3].

To identify the viruses affecting soybeans in different regions the soybean leaves with typical symptoms of viral infection were collected from every field in August 2010. Symptoms of soybean viruses include a mosaic pattern of light green/yellow and dark patches, distorted and wrinkled leaves, stunted plants, seed mottling, death of stems and petioles, and bud blight.

Separation of virus strains was performed by differential hosts. Differentiation among virus strains has been recognized on the basis of symptoms induced or the ability, or inability to infect certain species or varieties of plants.

The virus yield was obtained from systemically infected leaves 20 days after inoculation. Virus strains were purified as previously described [6].

Intracellular inclusion bodies in the epidermis of leaves of infected plants were detected after

fixation by Carnoy fluid (alcohol: ether: acetic acid = 6:3:1) or 3% trichloroacetic acid. After fixation preparations were washed in distilled water and stained with Giemsa stain for 25-30 minutes [2].

The investigations of soybean virus diseases all over the Ukraine regions were made by the enzyme-linked immunosorbent assay (ELISA) method and by electron microscopy.

Electron microscopic observations of purified viruses or leaf-dip preparations from infected soybean leaves were carried out using an electron microscope JEM 1400. Preparations were stained with 1% potassium phosphotungstate or 1% uranyl acetate.

Purified virus or plant sap extracts were evaluated using an indirect ELISA method with alkaline phosphatase using a diagnostic ELISA kit, available commercially from Agdia. Viral antigens were detected in leaf extracts of healthy or infected plants, as well as in preparations of purified virus. Absorbance values were recorded at 405 nm in BioTek ELISA reader.

Results and discussion. In 2010, virus diseases of leguminous crops in 3 ecological regions of Ukraine have been investigated. Infected plants in a soybean field displayed typical symptoms of viral infection such as curling of leaves, distorted and wrinkled leaves, deep leaf color and pale green or yellow mosaic, brownish necrosis along the leaf veins, or dwarfing (Fig. 1). In field studies it was found that the disease levels were very low and the degree of damage and the number of infected plants in different regions varied considerably. It depends largely on the soybean variety and growth conditions of the soybean plants. In Cherkassy region single virus plants were detected both in experimental and industrial fields. In Vinnitsa and Kyiv regions we observed a similar state of soybean disease level. This situation can be explained by the lack of perennial weeds that may be alternative hosts for the virus, because the infected weed is one of the important sources of SMV introducing into a soybean field; keeping of agricultural methods for soybean growth; good management strategies for a pest complex. During this period in the fields of Vinnitsa, the level of natural virus infection was about 20%, however almost 80% soybean plants of Oksana variety had typical symptoms of the viral disease. The spread of SMV in the field was evaluated by ELISA monitoring. The SMV titer in infected plants from different regions of Ukraine, as determined by enzyme-linked immunosorbent assay, was 4-6 times higher than in control samples from healthy soybean plants (Fig. 2). These data indicate the presence of high antigen concentration in soybeans with specific symptoms of viral infection: a mosaic, plant stunting, wrinkling, crimping the leaves, etc. At the same time using ELISA we did not detect SMV antigen in the plants of white pigweed (*Chenopodium album*), pea (*Pisum sativum* L.), fragrant rank (*Lathurus odoratus*), leguminous herbs, bean (*Phaseolus vulgaris)).* The main sources of viruses in the fields of Vinnitsa region were sowing wild soybean, which are used in breeding new varieties of soybeans and affected considerably the plants of some soybean varieties (cv. Oksana).

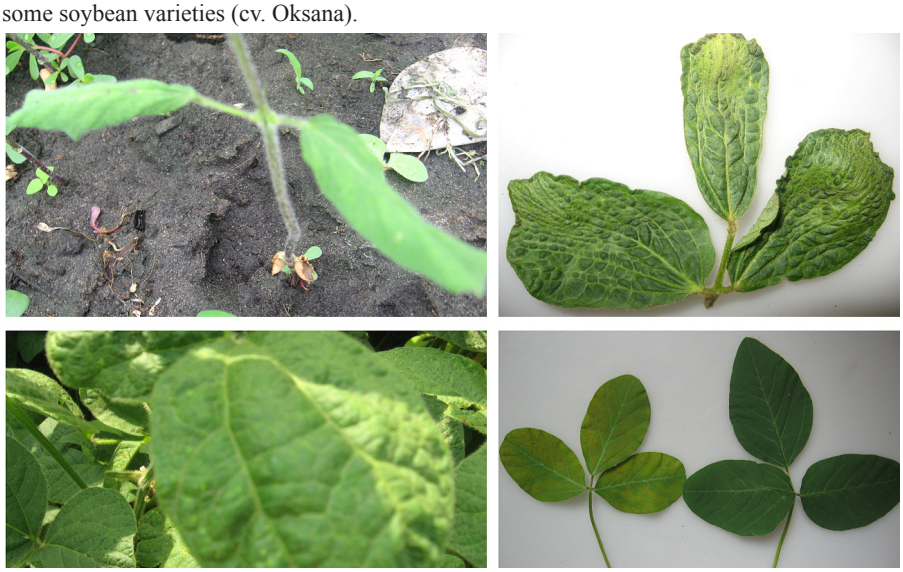

Fig. 1. Symptoms of soybean diseases in *Glycine soja L.*, produced by different virus isolates (field observations)

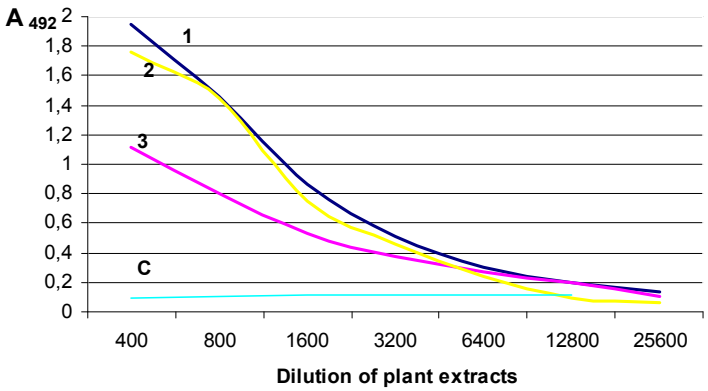

Fig. 2. Determination of the SMV by indirect ELISA method (leaf extracts of healthy (c) or infected (1-3) plants)

Ross [11] reported SMV isolates varied significantly in their pathogenicity to soybean selections. Host reactions depended on the soybean genotype and SMV strains. Most commercial soybean varieties are susceptible to SMV, so in the investigation various varieties of soybean were tested for susceptibility to SMV, namely: Monada, Themis, Smolyanka, Agata, Podolska, Oksana, Ustya, Kyivska-98, Angelica, Donka, Oriana and genetically modified soybeans. It was established that all tested varieties were susceptible to Ukrainian isolates of the virus and no varieties (including hypersensitive ones) resistant to infection of different virus isolates (from Vinnitsa, Cherkassy and Kyiv regions) were found among inoculated plants.

The presence of abundant inclusion bodies of typical morphology and localization is the aid to diagnosis in most hosts. This criterion is usually used to identify viruses. It is known that *Potyviruses* produce crystalline inclusions in leaf cells of both broad and common beans (*Vicia faba* L. and *Phaseolus vulgaris* L.) which have been readily detected with the light microscope [10]. The presence of crystalline inclusions in cells of *Vicia faba* L. and *Phaseolus vulgaris* L. leaves infected with different virus isolates was studied. Granular and/or crystalline cytoplasmic inclusions were detected in the epidermal tissues in both inoculated and young leaves, grown after infection (Fig. 3). Moreover, the inclusions found in the epidermis of plants infected with a purified virus or the sap of diseased plants did not differ and were identified as friable (amorphous, finely granular formation) that are localized not only in the perinuclear region, but also in other parts of the cell. The type of inclusions and their location can be indicative that the isolated viruses belong to *Potyviridae* family.

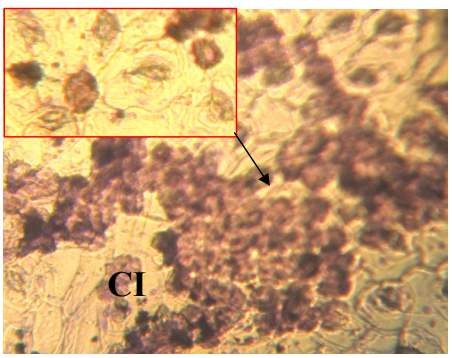

Fig. 3. Cylindcrical inclusions (CI) examined *in situ* with light microscopy in the epidermis of *Vicia faba* plants

The symptoms and ability to infect different plant species have been taken in account to identify the virus. Three virus isolates were studied by host range under greenhouse conditions. Reactions of the tested plants to infection are summarized in Table 1. Results from our studies on host range and symptomatology clearly indicated that biological properties of virus isolates from different regions were common and the most prevalent viruses were BYMV and SMV. They indicate that SMV and

BYMV occur together in the same plant (multiple infections). In studies the viruses were separated on the basis of host range (differential host method) and separated BYMV was used to further accumulation and maintaining in the greenhouse.

Table 1

Reactions of differential hosts to virus isolates from different regions of Ukraine*

Plant species	Virus characteristic symptoms	
	BYMV	SMV
Aizoaceae		
Tetragonia expansa L.	necrotic local lesions	–/–
Amaranthaceae		
Gomphrena globosa L.	local infection, necrotic local lesions with red border	–/–
Atriplex hortensis L.		local lesions on inoculated leaves
Ch. alba L.	–/–	local lesions
Ch. amaranticolor (Coste & A. Reyn.)	chlorotic local lesions with border, irregular systemic vein yellowing, leaf malformation	–/–
C. quinoa Willd.	chlorotic local lesions; not systemic	diffuse chlorotic local lesions
Lupinus luteus	necrotic local lesions	chlorosis, mosaic
Fabaceae		
Dolichos biflorus L.	–/–	local lesion
Phaseolus vulgaris L.	systemic yellowish mosaic, leaf curling, malformation, stunting, pods mottled	necrotic local lesions on inoculated attached primary leaves
Vicia faba L.	vein chlorosis, green/yellow mosaic, leaf distortion, downward curling	
Glycine soja L.	–/–	rolling or distorting mosaic, light green and yellow patches, distorted and wrinkled leaves, stunted plants, downward curling
Vigna sinensis L.		latent infection
Trifolium repens L., *Pisum sativum* L. (*Fabaceae)*, *Cucumis sativum* L. (*Cucurbitaceae*), *Petunia hybrida* Villm, *Nicotiana glutinosa* L., *N. rustica L* (*Solanaceae*) were unsusceptible to viruses.		

* - mechanical inoculation in the greenhouse condition

Purified virus was prepared from systemically infected leaves of *Phaseolus vulgaris* plants. Viruses of the *Potyvirus* genus are difficult to purify because of their tendency to aggregate, both end to end and side by side [12]. The average yield of virus was 3 mg (assuming an extinction coefficient 2.4) from 100 g of treated leaves. The absorbance ratio 260 nm /280 nm was 1.24-1.3 (corrected for light scattering). Electron micrographs of purified viruses showed flexible rod shaped particles. The majority of the particles measured between 700-760 nm. In particle size and morphology and in host reaction the virus isolates resembled BYMV, and they were not readily distinguishable from other *Potyviridae* (Fig. 4). The virus preparations had a proper degree of purification and concentration required to obtain a specific antiserum.

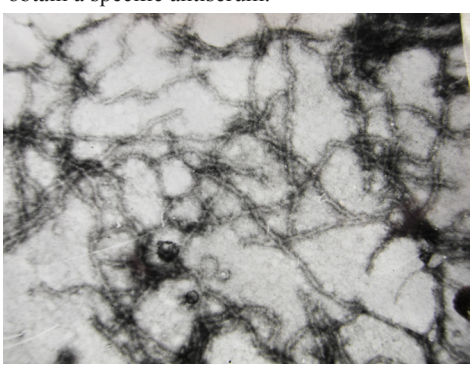

Fig. 4. Electron micrographs of BYMV. Magnification x 90 000

Soybean mosaic virus is currently not a production problem in Ukraine because the disease levels are very low. However, this virus disease is seed borne, and efforts must be made now to prevent the spread of this disease in seed sources. Another concern about SMV is dual infection with other viruses (BYMV), a common situation that increases the risk of yield loss and reduced seed quality.

For more accurate identification of viruses we are planning to explore the physical and chemical properties of the virions, the molecular weight of capsid protein and features of the viral genome. The purified virus is used as antigen to produce a specific polyclonal antiserum in rabbits and for further study of relationships between different soybean virus isolates in other agricultural regions of Ukraine.

Кириченко А.М., Краєва Г.В., Коваленко О.Г.

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ МСП, Д03680, Україна

ВІРУСНІ ХВОРОБИ СОЇ В РІЗНИХ РЕГІОНАХ УКРАЇНИ: БІОЛОГІЧНІ ХАРАКТЕРИСТИКИ ТА ІДЕНТИФІКАЦІЯ ЗБУДНИКІВ

Р е з ю м е

Проведено польові обстеження посівів сої на полях Черкаської, Вінницької та Київської областей щодо ураженності їх вірусними хворобами. Встановлено, що соя в цих регіонах уражена вірусами, основними серед яких є вірус мозаїки сої (ВМС) та вірус жовтої мозаїки квасолі (ВЖМК). Результати польових досліджень підтверджено методами світлової і електронної мікроскопії, а також за допомогою ІФА.

К л ю ч о в і с л о в а: *Glycine max* (L), потівіруси, вірус мозаїки сої (ВМС), вірус жовтої мозаїки квасолі (ВЖМК), поширення, ідентифікація.

Кириченко А.М., Краева Г.В., Коваленко О.Г.

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины,
ул. Академика Заболотного, 154, Киев МСП, Д03680, Украина

ВИРУСНЫЕ БОЛЕЗНИ СОИ В РАЗНЫХ ОБЛАСТЯХ УКРАИНЫ: БИОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ И ИДЕНТИФИКАЦИЯ ВОЗБУДИТЕЛЕЙ

Р е з ю м е

Проведены полевые обследования посевов сои на полях Черкасской, Винницкой и Киевской областей на поражаемость вирусными болезнями. Установлено, что соя в этих регионах поражена вирусами, из которых основными являются вирус мозаики сои (ВМС) и вирус жёлтой мозаики фасоли (ВЖМК). Результаты полевых исследований подтверждены методами световой и электронной микроскопии, а также с помощью ИФА.

К л ю ч е в ы е с л о в а: *Glycine max* (L), потивирусы, вирус мозаики сои (ВМС), вирус жёлтой мозаики фасоли (ВЖМК), распространение, идентификация.

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